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DEPARTMENT OF CLINICAL SCIENCE AND EDUCATION,
Karolinska Institutet, Stockholm, Sweden

THE PROPRIOCEPTION AND NEUROMUSCULAR STABILITY OF THE BASAL THUMB JOINT

NATHALIE MOBARGHA



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To my beloved ones.

ABSTRACT

OBJECTIVES

The basal thumb joint, or the first carpometacarpal joint (CMC1) is an enigmatic construction. The concavo-convex shape of the CMC1 and wide range of movement, allows for both precision and power grips and is particularly susceptible to the development of osteoarthritis. Previous theories regarding the development of CMC1 osteoarthritis have focused on the role of gender, excessive joint load and ligament laxity as causative factors. An emerging theory is the role of proprioception and sensorimotor functions that act to maintain adequate joint equilibrium. This concept constitutes the basis of this thesis with the intent on examining both healthy and osteoarthritic joints. Using microscopic, biomechanical and neurophysiological methods, we address the hypothesis that CMC1 holds proprioceptive and neuromuscular properties that are integral for proper thumb function.

METHODS

Patients with CMC1 osteoarthritis that were treated surgically with complete trapeziectomy and suspension arthroplasty were included. Two CMC1 ligaments were collected from these subjects: the anterior oblique ligament (AOL) and the dorsal radial ligament (DRL). Following immunofluorescent triple staining techniques using markers p75, PGP9.5 and DAPI, the ligamentous innervation and mechanoreceptor populations were analyzed (study I-II). In study III, biomechanical assessment of the role of isometric muscle load on CMC1 was performed by a FASTRAK® motion tracking device. In study IV, two intrinsic (the first dorsal interosseous muscle; DI, abductor pollicis brevis; APB,) and two extrinsic muscles (extensor pollicis longus; EPL, abductor pollicis longus, APL) were examined in healthy subjects using electromyographic (EMG) analysis during functional thumb tasks. Post-stimulus reactions following stimulation of the DRL were analyzed for each muscle and each thumb position.

RESULTS

The CMC1 ligaments investigated displayed a varying degree of innervation. AOL presented with little innervation and was mainly composed of incoherent connective tissue and scarce collagen fibers, whereas DRL presented with abundant innervation and well-organized collagen (study I). Unclassifiable mechanoreceptors were the most frequent type of receptor found in AOL and DRL. No significant difference in the innervation between the proximal and distal portions of these osteoarthritic ligaments was identified (study II). The first dorsal interosseous muscle (FDI or DI) produced the highest level of distal migration and the least dorsoradial translation of the base of the first metacarpal. APL was identified as the main destabilizer as it increases dorsoradial misalignment (study III). Following stimulation of the DRL, significant ($p < 0.05$) post-stimulus changes were found in all four muscles (DI, APB, APL, EPL) and positions tested. A mass inhibitory reaction was observed during tip pinch whilst key pinch produced rapid co-contractions. During palmar pinch a rapid inhibitory response was evoked in antagonistic muscles (study IV).

CONCLUSIONS

Ligaments from osteoarthritic patients displayed alterations in distribution and type of mechanoreceptors as compared to previous studies on normal joints. The results reinforce DRL's proprioceptive and stabilizing role for the CMC1 joint. Isometric load of CMC1 indicates that DI promotes joint congruency by reducing dorsoradial translation, whereas APL acts in an opposing manner as a destabilizing force. Ligamento-muscular reflexes were observed following the stimulation of DRL. Thus, the dorsoradial ligament, in addition to being the primary static stabilizer of the joint, also possesses proprioceptive qualities. The post-stimulus reactions detected in DI, APB, APL and EPL are indicative of protective ligamento-muscular pathways, which facilitate neuromuscular functions and the maintenance of joint stability.

CLINICAL RELEVANCE

Patients with CMC1 osteoarthritis constitute a large group. Their symptoms are of varying severity, where some can be addressed within the scope of primary care units, whilst others are in need of operative treatment. Patients with CMC1 osteoarthritis often present with impaired neuromuscular functions, reduced range of motion, weakness, joint and ligamentous pathology as well as pain, which affect their daily activities. Understanding the proprioceptive and neuromuscular characteristics of the basal thumb joint is therefore essential in deciphering the complex pathophysiology of the basal thumb joint.

Key words: basal thumb joint, trapeziometacarpal joint, carpometacarpal joint, ligament, proprioception, neuromuscular, osteoarthritis

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following scientific papers, which are referred to in the text by their roman numerals.

- I Mobargha N, Ludwig C, Ladd AL, Hagert E.
Ultrastructure and innervation of thumb carpometacarpal ligaments in surgical patients with osteoarthritis.
Clinical Orthopaedics and Related Research. April 2014, Vol 472, Issue 4.
- II Ludwig C*, Mobargha N*, Hagert E, Okogbaa J, Ladd AL.
* Shared first authorship
Altered Innervation Pattern in Ligaments of Patients with Basal Thumb Arthritis.
Journal of Wrist Surgery. In press.
- III Mobargha N, Esplugas M, Garcia-Elias M, Lluch A, Megerle K, Hagert E.
The Effect of Isometric Muscle Load on the Basal Thumb Joint: a Cadaveric Study.
Journal of Hand Surg (Eur) Vol. 2015 Aug 6.
- IV Mobargha N, Rein S, Persson KE J, Hagert E.
Ligamento-muscular reflex patterns following stimulation of a thumb carpometacarpal ligament: an electromyographic study.
Manuscript.

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LIST OF ABBREVIATIONS

ADD	Adductor pollicis
AOL	Anterior oblique ligament
APB	Abductor pollicis brevis
APL	Abductor pollicis longus
CMC1	First carpometacarpal joint
DAPI	49,69-diamidino-2-phenylindole
DI	First dorsal interosseous muscle (a.k.a FDI)
DRL	Dorsal radial ligament
EPB	Extensor pollicis brevis
EPL	Extensor pollicis longus
FDI	First dorsal interosseous muscle (a.k.a DI)
FPB	Flexor pollicis brevis
FPL	Flexor pollicis longus
HE	Hematoxylin and eosin staining
IP	Interphalangeal joint
IF	Immunofluorescence
MC1	First metacarpal
MCP	Metacarpophalangeal joint
OA	Osteoarthritis
OPP	Opponens pollicis
p75	Low-affinity nerve growth factor receptor p75
PGP9.5	Protein gene product 9.5
TMC	Trapeziometacarpal

1 THESIS AT A GLANCE

STUDY I

Ultrastructure and innervation of thumb carpometacarpal ligaments in surgical patients with osteoarthritis [1].

Are osteoarthritic CMC1 ligaments innervated with mechanoreceptors?

Material: Two ligaments, the anterior oblique ligament (AOL) and dorsal radial ligament (DRL), were harvested from patients with CMC1 osteoarthritis during trapeziectomy (fig. 1).

Methods: The ligaments were sectioned at a thickness of five μm and further analyzed using immunofluorescent triple staining techniques.

Conclusions: The CMC1 joint in osteoarthritic patients has ligaments, which are innervated with mechanoreceptors. DRL had the highest prevalence of mechanoreceptors.

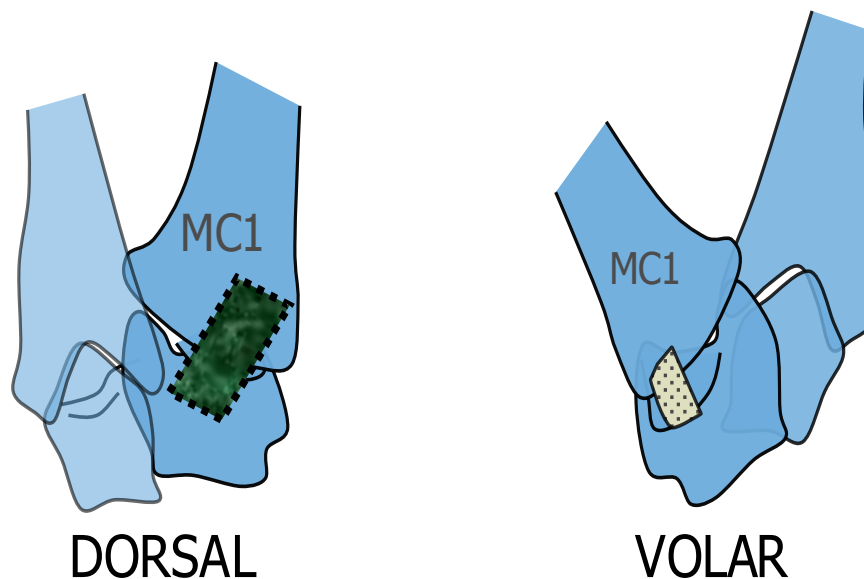


Figure 1. Illustration of dorsal and volar CMC1 ligaments. The DRL is composed of organized collagen fibers and reported with significantly ($p < 0.001$) more nerve endings and mechanoreceptors than the AOL. Courtesy of M Garcia-Elias, MD.

STUDY II

Altered innervation pattern in ligaments of patients with basal thumb osteoarthritis [2].

How are mechanoreceptors distributed in the CMC1 joint ligaments of osteoarthritic patients?

Material: Ligaments from patients with osteoarthritis.

Methods: Ligaments were marked for their proximal and distal ends respectively and immunofluorescent triple staining microscopy was performed.

Conclusions: The innervation of ligaments with no to mild signs of osteoarthritis differs from those of surgical patients with advanced osteoarthritis, in which a predominance of unclassifiable mechanoreceptors were detected (fig. 2).

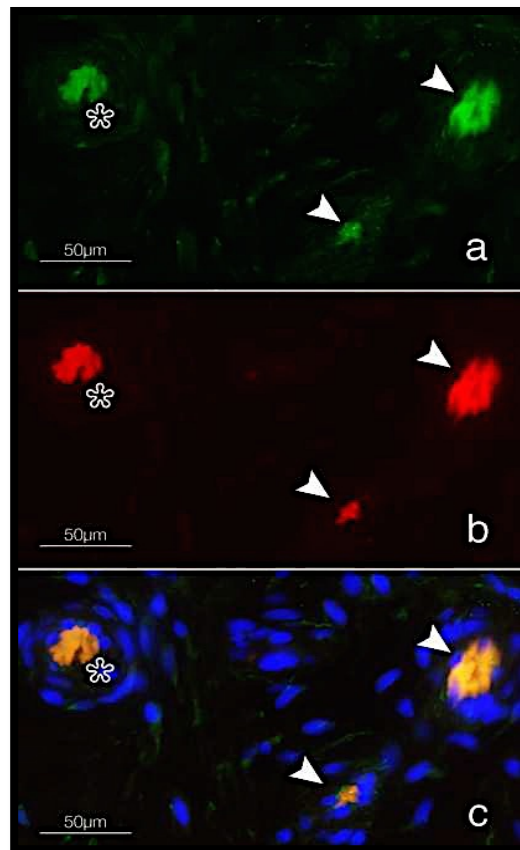


Figure 2. Unclassifiable mechanoreceptors. Two unclassifiable corpuscles are identified (arrowheads) and a transected arteriole () from an anterior oblique ligament stained with (a) p75 (b) PGP9.5 and (c) DAPI (blue). (Reprinted with permission from Ludwig C et al. Altered Innervation Pattern in Ligaments of Patients with Basal Thumb Arthritis. Journal of Wrist Surgery. 2015).*

STUDY III

The Effect of Isometric Muscle Load on the Basal Thumb Joint [3].

Do certain muscles exert a destabilizing effect on CMC1?

Material: Testing of ten cadaveric specimens without any signs of CMC1 joint osteoarthritis.

Methods: FASTRAK® sensors were used at the proximal end of metacarpals I and III and the distal radius to evaluate the effect of individual isometric muscle loading on the base of the thumb metacarpal relative to the radius and to the base of the middle finger metacarpal.

Conclusions: The first dorsal interosseous muscle (DI) has the most beneficial impact on the basal thumb joint. As such, strengthening of this muscle is recommended to patients with CMC1 osteoarthritis, or as a part of a pre- and post-operative rehabilitation strategy.

STUDY IV

Ligamento-muscular reflex patterns following stimulation of a thumb carpometacarpal ligament.

Is there a ligamento-muscular reflex pathway present between the DRL and the muscles (DI, APB, APL and EPL) acting on the CMC1 joint?

Material: 10 healthy participants with no prior hand trauma.

Methods: Ultrasound guided insertion of the DRL was followed by stimulation of the ligament while monitoring electromyographic activity the muscles during functional thumb tasks.

Conclusions: A ligamento-muscular reflex response is present in the CMC1 joint. Mass inhibitory response was evoked during tip pinch (fig. 3). During other tasks tested we observed co-contractions, which are suggested to contribute to joint stability.

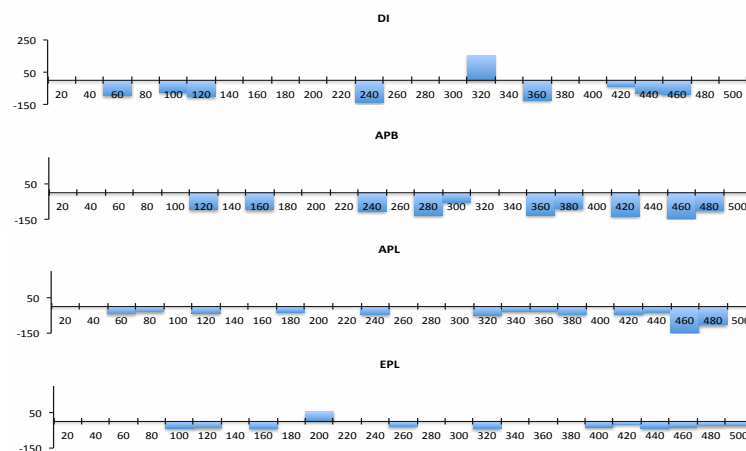


Figure 3. EMG amplitude for CMC1 muscles during tip pinch ($p < 0.05$). Illustration of significant changes ($p < 0.05$) in the amplitude (Y-axis) for all four CMC1 muscles for the time period (X-axis) 0-500 ms following stimulation of the dorsoradial ligament, divided into 20 ms time intervals.

2 INTRODUCTION

2.1 BACKGROUND

The human opposable thumb: get an evolutionary grip

The human opposable thumb is according to Aristotle, apart from the ability of speech and sense of logic, what sets us apart from other creatures [4,5].

The human thumb and its ability to perform fine tuned tasks as well as power grips (fig. 4) [6], has developed over the course of a million of years and several lineages, resulting in our capacity to construct and use advanced tools. Our simian ancestors were only capable of flexion and extension towards the palm of the hand and did not have the ability to oppose the thumb [7,8]. Thumb opposition is a primary motion in hand function and allows for eloquent and controlled grips and is determined by the thumb's ability to oppose against the other digits of the hand.

In present day and age, we are still dependent on the precision and complexity of the thumb machinery to function in our daily tasks. It has been suggested that the thumb accounts for 40% of our hand function [9]. This may explain the high occurrence of osteoarthritis of the thumb. In fact, thumb carpometacarpal joint (CMC1) osteoarthritis constitutes the most common site of elective surgery in the upper extremity. The radiographic prevalence of CMC1 osteoarthritis in persons older than 75-years is 40% for women and 25% for men [10,11].



Figure 4. Power grip (left) and precision grip (right). Napier acknowledged these two main grips as crucial for our evolution [6].

Consequently, hand disorders and in particular thumb dysfunction, is not merely a medical issue, but also a socio-economic matter. Regardless of the type of injury or loss of function in the upper extremity, the daily work and leisure of this group of patients is affected [12,13]. This causes major health care costs besides expenses attributed to their absence from labor [13-15].

2.1.1 Osteology

The term saddle-joint is occasionally used to describe the biconcavo-convex shape of CMC1 (fig. 5). To illustrate the shape of this joint even better, it has been likened to a "scoliotic horse with a saddle", where the metacarpal is facing sideways in the saddle [16]. This multifaceted joint allows for great modalities of motion including flexion, extension, adduction, abduction and combinations of these motions which yields complex movements such as radial and palmar abduction or adduction as well as retropulsion, and opposition [17]. However, complete CMC1 joint rotation is not possible due to the shape of the articular surfaces, just as a rider can't turn around freely in a saddle.

Previous research has established that the incongruent articular facets of CMC1 have a size difference of 34% when comparing the diameter of the first metacarpal (MC1) joint surface (16.03 ± 1.27 mm) to that of the distal trapezium (11.96 ± 1.32 mm) [18]. Due to this innate incongruency of CMC1, this joint is highly dependent on surrounding soft tissue, such as muscles and ligaments for its stability.

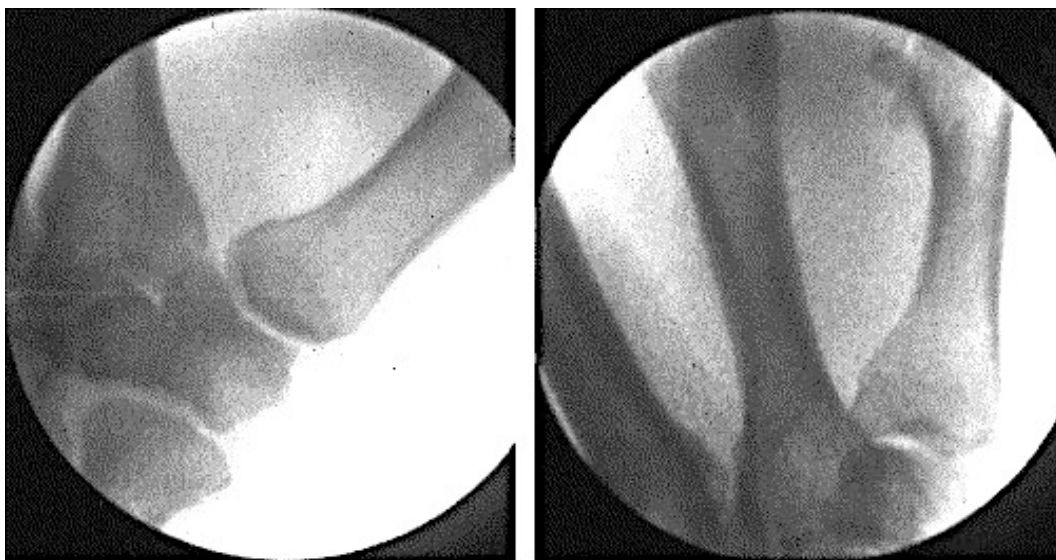


Figure 5. X-ray of the concavo-convex CMC1 joint. In the anterior-posterior view (left) the convexity of metacarpal is noticeable while the trapezium is concave. Inversely, in the lateral view of the CMC1 joint the trapezium is convex whilst the metacarpal is concave. (From Edmunds JO. Traumatic dislocations and instability of the trapeziometacarpal joint of the thumb. Hand clinics. 2006, 22: 365-92; with permission to reprint).

2.1.2 Muscles

Besides static restraint from surrounding ligaments, a joint is dependent on the dynamic control from muscles that act on the joint to maintain stability and congruency [19,20].

The following muscles serve as primary CMC1 muscles: musculus abductor pollicis brevis (APB), flexor pollicis brevis (FPB), opponens pollicis (OPP), flexor pollicis longus (FPL), adductor pollicis (ADD), abductor pollicis longus (APL), extensor pollicis brevis (EPB), extensor pollicis longus (EPL) and the first dorsal interosseous muscle (DI). These muscles may differ in their characteristics due to anatomical variations, trauma or as a consequence of congenital disorders. As muscular biomechanics are often altered following nerve injury or osteoarthritis [21], it is of particular interest to understand their course of action in normal conditions.

Intrinsic muscles

The intrinsic thumb muscles are APB, ADD, FPB, OPP and DI and are innervated by the median, radial and the ulnar nerves.

APB primarily abducts and flexes MC1. This muscle is primarily innervated by the median nerve (95%), but it can also be innervated by the ulnar nerve (2.5%) or by both nerves (2.5%) [8]. This muscle arises by part from the scaphoid, trapezium and the transverse carpal ligament and attaches to the radial sesamoid bone and the metacarpophalangeal joint (MCP1) [8,22]

ADD consists of two heads and between these heads lays the ulnar nerve, which innervates this muscle [22,23]. As its name implies, this muscle is an adductor of the MC1.

DI has a deep head, which arises from the volar aspect of MC2, and a larger superficial head that originates from MC1 medially [24]. This bipennate muscle is capable of flexing as well as abducting the MCP1 [25,26]. The DI is often recognized as a muscle innervated by the ulnar nerve, however it has also been suggested that this muscle presents with considerable variance in its innervation [27].

FPB arises from the transverse carpal ligament and attaches to the MCP1 and the radial sesamoid [8]. This muscle is a flexor of the proximal phalanx and MCP1. It is composed of a superficial head, innervated by the median nerve, and a deep head innervated by the ulnar nerve. However, there are many variations of the innervation of FPB [23,28,29].

OPP pronates and flexes the thumb. OPP is another thumb muscle with varying innervation; either by the median nerve (83%), the ulnar nerve (10%) or by both of the nerves (7%) [8,29].

Extrinsic muscles

APL is located in the first extensor compartment, sometimes accompanied by single or multiple auxiliary slips and receives its nerve supply from the posterior interosseous nerve [30,31]. While the main tendon inserts on the radial base of the MC1, other slips may insert on the volar CMC1 and other carpal bones. Although its name implies an abduction function, the primary function is one of combined extension and slight abduction of the MC1. An old term for this muscle was "extensor ossis metacarpi pollicis", which in fact is more true to its function [16].

EPB is also located in the first extensor compartment and is likewise innervated by the posterior interosseous nerve. Its action on the CMC1 is closely related, albeit weaker, than that of the APL. In addition, it's a primary extensor of the MCP1.

EPL runs as a single tendon in the third extensor compartment and is also innervated by the posterior interosseous nerve. The EPL is an extensor of the IP joint as well as an important thumb adductor.

FPL is innervated by the anterior interosseous nerve and flexes the IP joint. FPL, sometimes observed with an auxiliary head, originates from the anterior aspect of radius and attaches to the base of distal phalanx of the thumb [8].

2.1.3 Ligaments

Because of the characteristic mobility and configuration of CMC1, this joint is highly dependent on proper ligamentous support to maintain stability as well as flexibility throughout all ranges of movement. A number of publications have investigated the intricate ligamentous apparatus that supports the CMC1 [8,18,32,33]. The number of CMC1 ligaments described ranges from three [34] to as many as sixteen [33]. The primary ligaments of CMC1 are the dorsal ligaments and the volar "beak" ligament (AOL), which will be further discussed below (fig. 6).

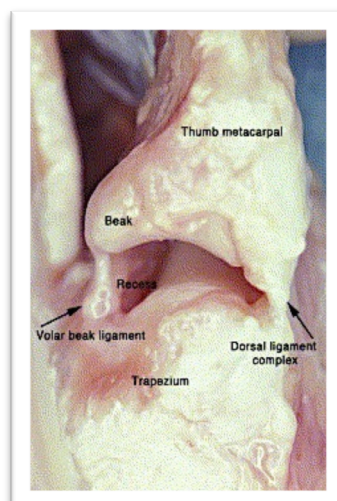


Figure 6. The volar beak ligament, also known as the anterior oblique ligament (AOL), arises from the volar side of MC1 and inserts to the volar aspect of the trapezium. The dorsal ligament complex arises from the tubercle of trapezium and attaches to the thumb base. (From Edmunds JO. Traumatic dislocations and instability of the trapeziometacarpal joint of the thumb. Hand clinics. 2006, 22: 365-92; with permission to reprint.)

The dorsal ligaments

The dorsal radial (DRL), dorsal central and posterior oblique ligaments constitute the dorsal ligament complex. These ligaments originate from the tubercle of the trapezium and attach at the thumb base (fig. 7). The dorsal ligaments are composed of distinct collagenous bundles that contain nuclei and also possess proprioceptive qualities as demonstrated by the presence of mechanoreceptors [1,35-37].

The debate whether the dorsal ligaments are the primary CMC1 stabilizers has gained prominence in the past decade. Many argue that the dorsal group of ligaments is in fact the primary stabilizer of CMC1 and not the thin volar ligaments [38-42].

Present evidence suggests that the dorsal ligaments constitute the thickest, strongest and most robust group of the CMC1 ligaments [36,43]. The dorsal ligaments support the CMC1 joint throughout rotational forces as well as dorsal dislocations [43-47].

The dorsal ligaments are central during the screw home torque mechanism. This mechanism is central in the prevention of dorsal subluxation during the closing stage of thumb opposition [40,43]. In addition, the dorsal ligaments are essential to counteract and stabilize the large compressive forces applied at CMC1 during power grips and power pinch [43].

Clinically, in cases where the dorsal ligaments are damaged, such as in CMC1 dislocations or following Bennett's fracture, the overall stability of CMC1 is jeopardized and may cause joint dislocation, even if the volar ligament is undamaged [43,44].

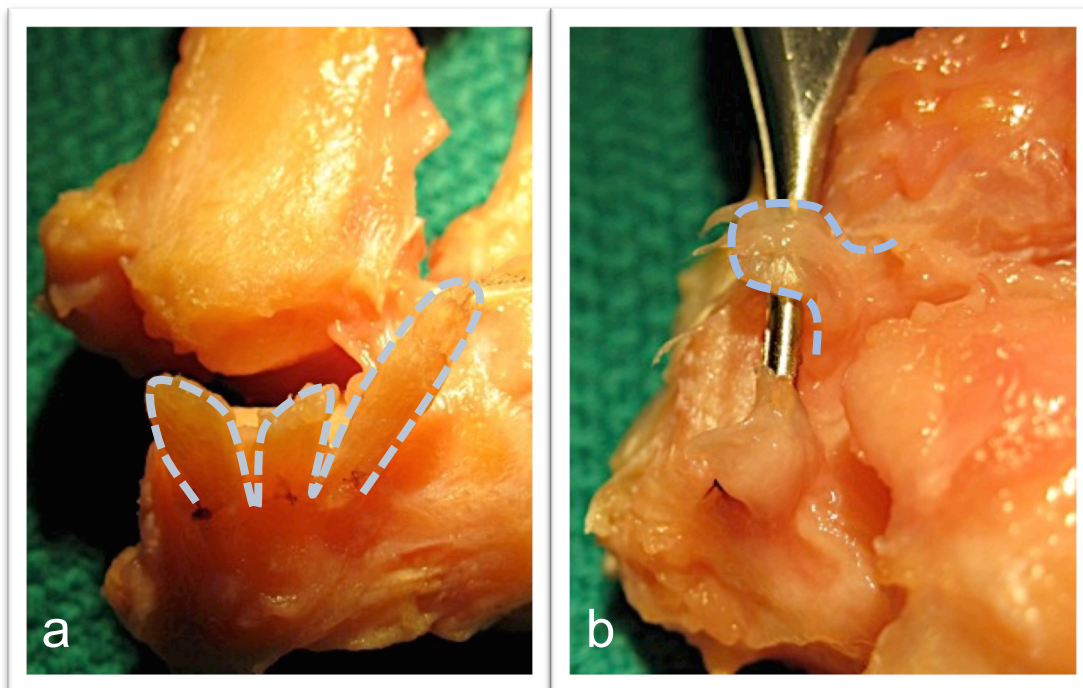


Figure 7. CMC1 ligaments. Two of the principal CMC1 ligament structures: a.) the dorsal ligaments and b.) AOL. Note that the AOL is thin and practically semitransparent.

The anterior oblique ligament

The AOL, also known as the volar or palmar “beak” ligament, is a thin ligament that originates from the volar side of MC1 and attaches to the volar aspect of the trapezium (fig. 7). Formerly considered the primary ligament in CMC1 stability [34], existing research now recognizes that the AOL is significantly thinner, weaker and does not contain mechanoreceptors or organized collagen fibers with nuclei, unlike the dorsal ligaments [1,35-37,43]. The AOL is not crucial for the screw home torque mechanism, as this ligament remains lax during screw home torque and does not inhibit any tendency of dorsal subluxation [43]. This is further implicated in cases where the AOL is injured, in which no major destabilization of CMC1 is noted, unless there is any concurrent damage to the dorsal structures [43].

2.1.4 Innervation

The hand and particularly the thumb accounts for a large section in the neural mapping of motor and somatosensory cortex (fig. 8). This well-recognized schematic depiction of the cerebral cortex, was first made by an illustrator named Mrs. Cantile in 1937 based on the research of Penfield and Boldrey [48]. This graphic representation is based on muscle contractions found in a specific body region, which are evoked following electrical stimulus of the corresponding region in the cerebral cortex [49].

Although extensive research has been carried out since then to examine the bone morphology, ligament functions and muscular dynamics of CMC1, the innervation of this complex joint needs further attention. The CMC1 joint is particularly difficult to assess as the median, radial as well as the ulnar nerve innervate the many muscles acting on the joint. In addition, there is no real consensus regarding the innervation of the joint itself. The CMC1 has been proposed to be innervated by branches from the radial and median nerve [50], whereas others suggest the lateral antebrachial cutaneous nerve to innervate the joint as well [51]. Additionally, more novel studies also propose the ulnar nerve to contribute to the nerve supply of this complex joint [52].

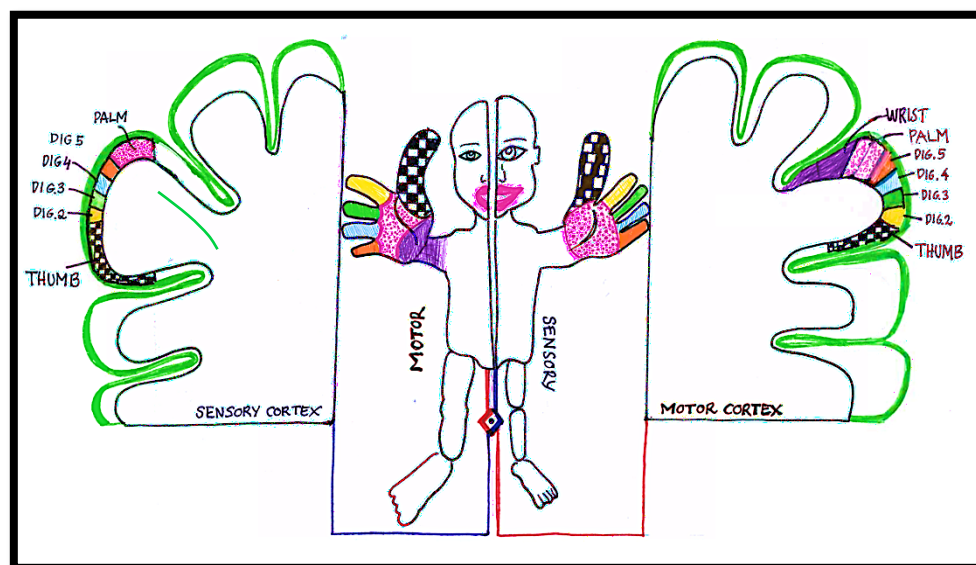


Figure 8. Graphic representation of cerebral cortex. Note the proportionally large area that represents the hand and especially the thumb, in both the motor and sensory cortex. Both sensory input and motoric output tracts cross the midline (blue/red). Courtesy of Mobargha A.

2.2 PROPRIOCEPTION AND NEUROMUSCULAR MECHANISMS

Research on the subject of joint stability has previously been restricted to the matters of muscles, ligaments and joint characteristics. However, evidence supports the notion that sensory input from ligaments, muscles, skin and joints is central in the maintenance of joint stability [53,54].

Neuromuscular control is the continuous muscular adaptive response to a motion, for the purposes of preserving joint stability and accuracy of motion. The physiological basis of neuromuscular control is founded on sensory afference from e.g. receptors in ligaments and tendons and the central integration of this proprioceptive input [53,54]. If any part of this integral construction is injured, whether it is the muscles, joint, ligament or nerves, the management and adaptation of physiological load upon the joint and adaptation to on-going movement may be inaccurate. Consequently, this may initiate and lead to muscle weakness, pain, subluxation and ultimately osteoarthritis [55,56].

2.2.1 Mechanoreceptors

Afference from sensory neurons in ligaments and tendons are important for neuromuscular control as they can impact muscle action [53,54]. There are different types of sensory neurons; large myelinated, fine myelinated or unmyelinated [57].

The larger myelinated axons, also known as mechanoreceptors, are highly receptive to mechanical stimulus, such as pressure or strain. The fine axons are primarily pain sensors and terminate as free nerve endings. These thin myelinated axons, are also recognized as fast A δ -fibers, and respond to noxious input. The C-fibers, also known as unmyelinated axons, are slow fibers that take part in inflammatory mechanisms by e.g. the release of inflammatory substances such as substance P [57].

The different types of sensory neurons and their characteristics, based on Freeman and Wyke's classification, are further described below[58].

Ruffini ending (class I)

This mechanoreceptor detects changes in velocity and amplitude and senses changes in joint position. The Ruffini ending is a slowly adapting mechanoreceptor with a low threshold. It is typically 50-100 μm and partially encapsulated and often seen with branches and bulbous endings.

By using the triple staining technique, PGP9.5 stained for the central axon and terminal nerve branches, whilst p75 stained for the capsule that was frequently incomplete.

Pacini corpuscle (class II)

The Pacinian corpuscle is a mechanoreceptor with low threshold. This mechanoreceptor rapidly detects and adapts to deceleration and acceleration. This corpuscle is either round or oval, ranges from 20 to 50 μm and is characterized by a thick lamellar capsule. Its central axon is visualized by PGP9.5 and the thick perineurial capsule by p75.

Golgi like receptor (class III)

The Golgi like receptor is also a rapidly adapting mechanoreceptor but with high threshold. It detects extreme ranges of motion and is characteristically spherical, measures > 150 μm and is partially encapsulated. It is recognized by groups of

arborizing and terminal nerve endings. PGP9.5 stains for the terminal nerve branches and p75 stains for the incomplete capsule.

Free nerve endings (class IV)

Free nerve endings can be classified as either fast A δ -fibers or slow C-fibers. The mutual and typical features of the free nerve endings are that they respond to nociceptive and noxious input and are involved in inflammatory activities. They can be found either in groups or as single nerve fibers with varicose appearance. Their axons stain for PGP9.5.

Unclassifiable receptors (class IV)

This heterogeneous class of receptors varies in their size and level of encapsulation. They often present with an incomplete capsule stained for by p75. Although they have previously been described [59,60], their exact neurophysiological functions and traits are still unknown and need further description.

2.3 OSTEOARTHRITIS

Osteoarthritis is a chronic disease with multiple etiologies, symptoms and treatments where the disease progression involves a complex chain of biochemical processes [8,61]. Some of the causes that are associated with the onset of osteoarthritis are previous trauma, increasing age, female gender, and ligament laxity due to idiopathic disease or hormonal changes associated with e.g. obesity [11,62-64]. Typically, this disease debuts amongst middle-aged women with pain as primary symptom [65].

Historically, osteoarthritis has been described as a disease restricted to the bone and cartilage due to abnormal biomechanical loads. More recent developments in this field have led to a description of a joint as a *synovial organ* where any component of that organ, such as its ligaments, muscles, nerves, cartilage or bone, may contribute and be engaged in the onset of osteoarthritis if compromised (fig. 9) [21]. Therefore, it is likely that osteoarthritis may still develop even in cases where the biomechanical joint load is normal, but where the other joint supportive tissues are impaired [56].

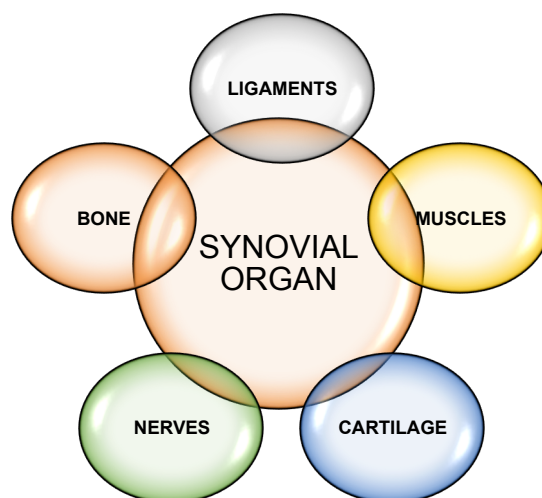


Figure 9. Osteoarthritis is not only a matter of bone and cartilaginous pathology. A joint should be regarded as a synovial organ, where injury to any of its components may be a part of the osteoarthritic process.

In light of recent events in the field of osteoarthritis, it is becoming difficult to ignore the existence and importance of neuromuscular and proprioceptive functions and how the failure of these mechanisms may contribute to onset of osteoarthritis [54,66,67]

This may also explain why radiographic findings do not always correlate with the clinical presentation of the disease. This is especially true for osteoarthritic changes in the soft tissues, which are not visualized through a classic X-ray [68]. Ligamentous changes are often found during osteoarthritis and are frequently observed in the small joints of the hand [56,69-71].

Nevertheless, the radiographic staging of CMC1 osteoarthritis proposed by Eaton and Littler is frequently used to describe some of the osteoarthritic CMC1 changes that can be visualized on an X-ray (fig. 10) [72,73]. Though osteoarthritis is a chronic disease, often found with radiographic deterioration, some patients remain in a constant stage, with no progressive joint disfiguration of their disease [74].

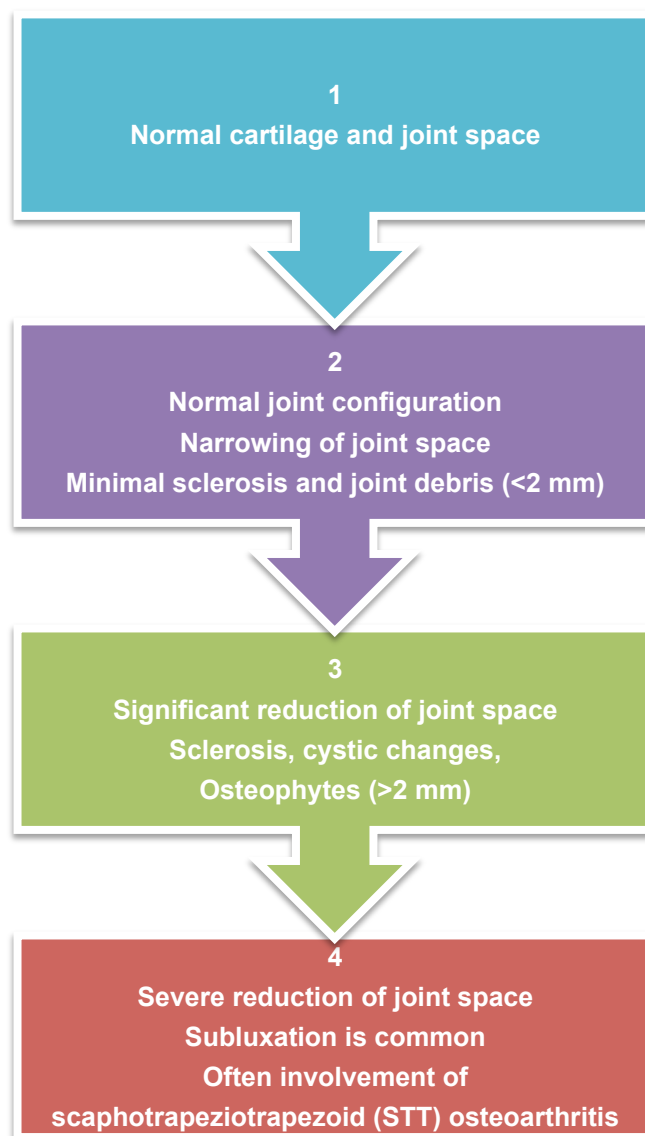


Figure 10. Radiographic staging of CMC1 osteoarthritis based on Eaton and Littler's classification [73].

2.3.1 CMC1 Osteoarthritis

The inherent incongruency of the CMC1 joint makes it prone to osteoarthritis and particularly dependent on its surrounding soft tissue for normal function. Therefore, soft tissue imbalance often accompanies CMC1 osteoarthritis. Regardless if these changes are pertained to ligamentous pathology or due to muscular deficiency, the global equilibrium of the CMC1 joint will be affected. Changes of the ligament structure, such as increased laxity and/or thickening of the ligament, may impact the onset of osteoarthritis due to the subsequent biomechanical changes [71,75].

Recent evidence suggests that ligamentous osteoarthritis can be described as its own stage in CMC1 osteoarthritis and has been suggested as a precursor of the disease [70,73,76]. Hormonal changes following menopause most likely contribute to joint laxity and may impact collagenous tissue. This may explain why females are more predisposed to CMC1 osteoarthritis [8,11].

Patients with CMC1 osteoarthritis often suffer from functional limitations, pain, joint instability or joint stiffness and even subluxation in more advanced stages (fig. 10) [77-79]. This subluxation manifests as a dorsoradial translation of the base of the MC1 relative to the CMC1. In the final stages, this subluxation is further accompanied by a secondary contracture of the adductor muscle, rendering the thumb metacarpal fixed in adduction toward the index metacarpal.

The role of joint innervation, neuromuscular control and proprioception in this pathophysiological process has yet not been illuminated.

3 AIMS OF THE INVESTIGATION

The general aim of this investigation was to explore and assess the proprioceptive and neuromuscular characteristics of the CMC1 joint. Specifically, the aims of this investigation were as follows:

- I. To investigate CMC1 ligaments in patients with osteoarthritis and analyze if they possess neural properties besides evident stabilizing functions.
- II. To examine the intraligamentous innervation of osteoarthritic patients and evaluate if it differs from normal subjects.
- III. To analyze the role of the muscles that act on the CMC1 with regard to promotion or prevention of joint instability.
- IV. To investigate if a CMC1 ligament has the ability to impact CMC1 controlling muscles through ligamento-muscular reflexes, and what role these reflexes may have in proprioception and neuromuscular joint control.

4 MATERIAL AND METHODS

4.1 ETHICAL CONSIDERATIONS

All studies performed have been granted ethical approval and were handled according to ethical guidelines and regulations. All participants were included following written and oral information and consent.

4.2 MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL PROCEDURES

Study I-II

The AOL and DRL were obtained from patients with advanced CMC1 osteoarthritis during trapeziectomy and suspension arthroplasty. These ligaments were selected based on their acknowledged importance in stabilizing CMC1 as well as the fact that they present with the highest mean difference of ligamentous innervation in normal subjects [35,36,46].

We used hematoxylin and eosin staining (HE) [80] for structural visualization of ligament histology. In addition, we employed triple staining techniques to visualize and distinguish nerves and mechanoreceptors from arteries and collagenous tissue.

4.2.1 Immunohistochemistry

The ligaments were sectioned in five mm width and five μ m thickness. In addition, ligaments were labeled for their proximal and distal insertions respectively. The slides were further prepared for immunohistochemistry (see studies I and II for detailed description).

Control staining

Both positive and negative control stains were performed. The negative control stains consisted of elimination of primary antibodies, resulting in loss of specific stains. For positive controls, staining was performed on cadaveric nerve tissue (median nerve samples), verifying proper staining of neural contents.

4.2.2 Primary antibodies

We used rabbit anti-protein gene product 9.5 (PGP9.5), and p75 rabbit anti-nerve growth factor receptor (p75) as primary antibodies.

PGP9.5 is a neuronal marker involved in the signaling of cell differentiation, cell death, neuronal growth and migration. All mammals have this protein, which is found in the neuronal cytoplasm and is particularly expressed in metabolically active areas. This primary antibody operates as a general nerve marker and as such labels axons of nerve fascicles and nerve corpuscles and their terminal branches. Therefore, this primary antibody is essential in the identification of mechanoreceptors, such as the Ruffini corpuscle and free nerve endings.

p75 is important as primary antibody due to its ability to detect nerve fascicles and their perineurial cells, and the capsule of nerve corpuscles. This antibody is crucial for detection of the Pacinian corpuscle by staining for its perineurial layers.

4.2.3 Secondary antibodies and nuclear labeling

Detection and amplification of primary antibodies was performed using goat anti-rabbit Alexa Fluor 488 for p75 and goat anti-rabbit Alexa Fluor 647 for PGP9.5.

In addition, we used 49,69-diamidino-2- phenylindole (DAPI) to label nuclei and to distinguish ligamentous tissue from nerves and vascular structures.

4.2.4 Immunofluorescence imaging

The immunohistochemical slides were systematically visualized and analyzed by a fluorescence microscope set at multidimensional acquisition to achieve full immunofluorescent array. First, the sections were imaged at larger magnification (x20) to identify p75 or PGP9.5 immunofluorescence (IF) and thus regions of interest. Following this, higher magnification (x40) was used to analyze details of the IF regions.

4.2.5 Morphological analysis

The innervation of the ligaments was graded using semi-quantitative analysis (table 1).

The classification of the microscopic ligamentous innervation was based on an adapted version of the classification of mechanoreceptor (table 2) first proposed by Freeman and Wyke [58]. This type of morphological classification of ligaments and their microscopic innervation has previously been applied to other studies of human ligaments [35,81,82].

ORDINAL GRADING SYSTEM FOR QUANTIFICATION OF MECHANORECEPTORS	
+++	Numerous nerve fascicles and mechanoreceptors
++	Solitary nerve fascicle and mechanoreceptor present
+	Nerve fascicle present, but no mechanoreceptors
-	No nerve fibers, nerve fascicles or mechanoreceptors identified

Table 1. An ordinal grading system was used to quantitatively evaluate the degree of innervation of ligaments.

Class	Name	Neurophysiological traits	Role in joint function	Appearance	Immunoreactive patterns
I	Ruffini	Low threshold Slowly adapting	Changes in velocity and amplitude Joint position sense	50-100µm Partially encapsulated Sprouting nerve branches with bulbous ending	Central axon (PGP9.5) Terminal nerve branches (PGP9.5) Incomplete capsule (p75)
II	Pacinian Corpuscle	Low threshold Rapidly adapting	Acceleration Deceleration	20-50µm Round/oval Thick lamellar capsule	Central axon (PGP9.5) Thick capsule (p75)
III	Golgi like receptor	High threshold Rapidly adapting	Extreme ranges of motion	> 150µm Spherical Partial encapsulation Groups of arborizing and terminal nerve endings	Terminal nerve branches (PGP9.5) Incomplete capsule (p75)
IV	Free nerve endings	Fast Aδ-fibers Slow C-fibers	Noxious and nociceptive input Inflammatory activity	Varicose appearance In groups or single fibers	Axon (PGP9.5)
V	Unclassifiable	Unknown	Unknown	Variable size and encapsulation	Incomplete capsule (p75)

Table 2. Mechanoreceptors in ligaments based on Freeman and Wyke's classification [58], modified by Hagert E [83] (Reprinted with permission from Hagert E 'Wrist Ligaments—Innervation Patterns and Ligamento-Muscular Reflexes', PhD thesis, Karolinska Institutet, 2008).

4.3 BIOMECHANICAL ASSESSMENT

Study III

In this study, fresh frozen human cadaveric hands and forearms with no signs of CMC1 osteoarthritis were used. X-rays of the specimens were taken prior to the experiments to exclude osteoarthritis.

4.3.1 Preparation of specimens

Specimens were thawed at room temperature. Following this, the distal third of the forearm and the metacarpophalangeal joints to digits 2-5 were transected.

The thumb was preserved, but soft tissues other than the thenar tendons and muscles, the CMC1 ligaments, flexor and extensor retinaculi, carpal ligaments and tendons of the extrinsic and intrinsic muscles, were removed.

Pins were used to mark the insertions and origins of the intrinsic thenar muscles. A 2.4 mm Steinmann pin was drilled through the third metacarpal intramedullary and another pin was positioned through the first interphalangeal joint, to ensure control of the CMC1 joint. Radius and ulna were also fixed to the jig by pins stabilizing the carpus and forearm. The thumb was positioned in neutral position and the specimen was firmly attached to a jig, especially designed and used for the purposes of biomechanical assessment.

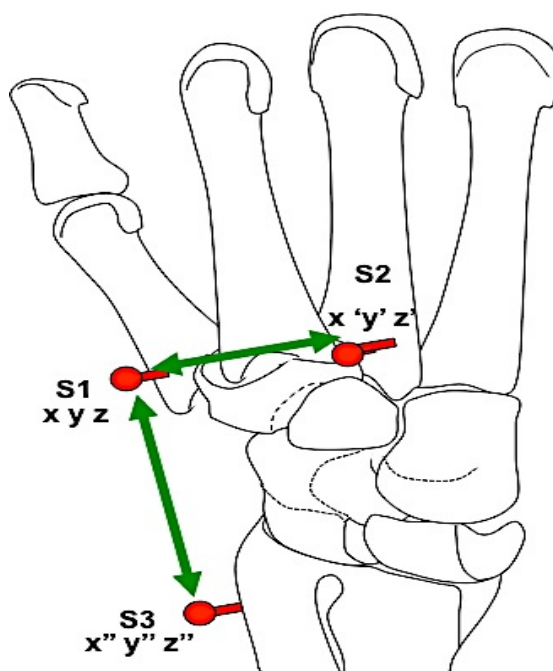


Figure 11. Measurements were obtained from sensors S1, S2 and S3 respectively. Courtesy of M Garcia-Elias, MD.

4.3.2 Biomechanical analysis

FASTRAK® three-dimensional motion tracking system (figs. 11 and 12) was used to identify spatial changes. Three sensors (S1, S2 and S3) were inserted into the base of the first and third metacarpals (S1 and S2, respectively) and a third sensor (S3) positioned on the dorsal aspect of the distal radius, 5 cm proximal from the CMC1. This system produced co-ordinates x, y and z which could then be used for analysis of three-dimensional spatial changes between the sensors. The intrinsic (APB, APL, OPP, DI) and extrinsic (FPL, APL, EPB, EPL) muscles acting on CMC1 were investigated individually.

Simulation of muscle tone was achieved by attachment of nylon strings to the insertion of the tendons and by loading them with 1N. Next, attachment of weights proportional to every muscle's physiologic cross-sectional area were used to obtain isometric conditions. The system was reset after analysis of each muscle to minimize error of measurement. To reaffirm the pre-experimental radiographs, each joint was opened and macroscopically assessed after finalization of the experiment, confirming that there were no signs of osteoarthritis.

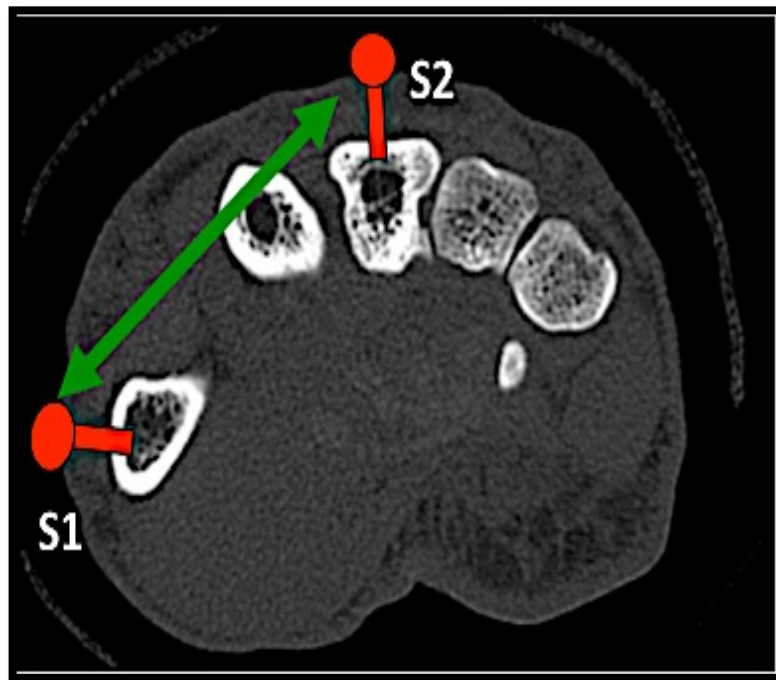


Figure 12. Illustration of sensor placement of sensor 1 (S1) and sensor 2 (S2). The distance between sensors S1 and S2 (green arrow) quantifies the horizontal displacement. Changes in this distance are representative of the movement of MC1 to MC2 as a result of isometric loading of CMC1. Positive values suggest that MC1 moves away from MC2, whereas negative values signify approximation of MC1 to MC2.

4.4 ELECTROMYOGRAPHIC PROCEDURES

Study IV

Subjects enrolled in this study were all healthy, with no signs of CMC1 osteoarthritis or dysfunction, nor prior wrist or hand trauma. Four primary CMC1 muscles, of which two were intrinsic (DI, APB) and two were extrinsic (APL, EPL), were investigated. The ligamento-muscular relation between DRL and the four muscles was analyzed following electrical stimulation of DRL by the means of electromyography (EMG).

Experimental protocol

Participants were seated in a chair with the arm and shoulder in resting neutral position and slight flexion of the elbow. The skin was cleansed with alcohol and shaved when necessary.

Ultrasound procedures

The muscle bellies of DI, APB, APL and EPL were first identified clinically, and then confirmed using an ultrasound system equipped with a transverse 8-MHz transducer (Esaote My Lab Five, Biomedica Ltd.). A 0.35 mm/diameter needle electrode was inserted into each muscle. As a positive control, EMG signals on the oscilloscope were observed during voluntary muscle activity to further ensure correct placement of electrodes.

Ultrasound was used to identify DRL and ensure correct placement of the stimulation electrode (fig. 13). Following visualization of DRL a 0.5 mm puncture needle was placed into the ligament and a hooked fine-wire electrode was inserted into DRL via the puncture needle. The fine-wire electrode was equipped with a hook to ensure firm placement into DRL so that the puncture needle could be removed without electrode dislocation. A reference electrode was placed distally from the stimulation electrode on the dorsal aspect of the hand. Following this the electrodes were connected to a 4-channel electromyograph.

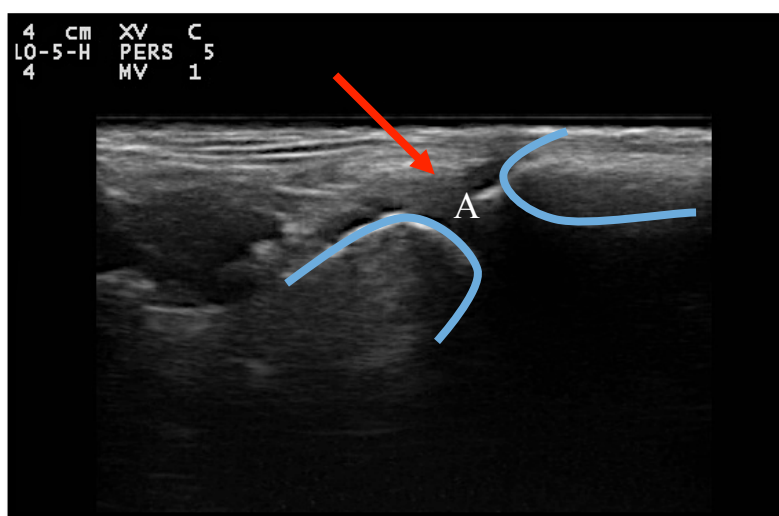


Figure 13. Identification of DRL by ultrasound. The stimulation electrode was inserted into the DRL (A).

Stimulation procedures and electromyographic analysis

The sensory threshold was determined by the input of a series of 200 Hz pulsations to DRL, each 1 ms long. The value obtained set the foundation for the experimental threshold, which was set at 2 times the sensory threshold but always below the pain threshold. EMG recordings began 100 ms before stimulation (pre-stimulus or t_1) of the DRL, until 500 ms after stimulation (post-stimulus or t_2).

Positions tested

The EMG activity after stimulation of the DRL was examined during the functional CMC1 thumb tasks tip pinch, key pinch and palmar pinch (fig. 14). The positions were performed during isometric conditions and at submaximal force to avoid muscle fatigue. To obtain this precondition, the maximum strength in each position was assessed using an intrinsic meter, or pinch gauge. The volunteers were subsequently instructed to use only 25% of their maximal force for each of the positions.

As a negative control, the DRL was stimulated when the four muscles were relaxed and the thumb in neutral position, to ensure that this did not elicit any signs of EMG activity.

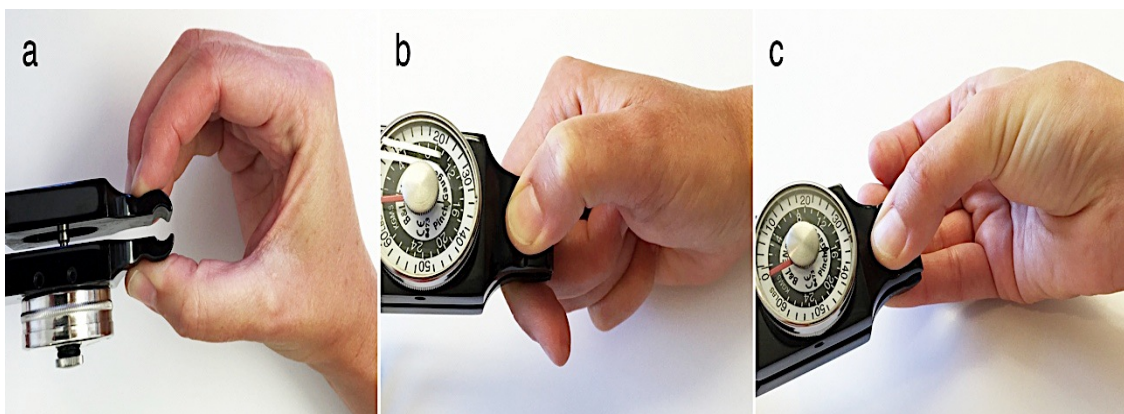


Figure 14. CMC1 positions investigated. Subjects were instructed to use only 25% of their maximal force for a.) tip pinch b.) key pinch and c.) palmar pinch to avoid muscle fatigue

5 RESULTS

5.1 IMMUNOHISTOCHEMICAL STUDIES

Study I-II

Previously established grading systems [35,81,82] were implemented to perform a semi-quantitative assessment of the innervation of ligaments investigated.

5.1.1 Structural differences

Study I

HE stain was used to examine the possible existence of nuclei and the microscopic structure of the ligaments. The HE staining (fig. 15) revealed distinct differences in the structural appearance of AOL compared to DRL. Whereas AOL was mostly composed of disordered connective tissue, DRL presented with well-organized collagen strands. This observation is in agreement with previous studies made on CMC1 ligaments with no signs of osteoarthritis [36].

DAPI labeling (fig.16) further exposed significant structural changes between AOL and DRL. Visualization using DAPI displayed presence of nuclei in DRL's well-organized collagenous network. On the contrary, AOL lacked nuclei (fig. 16), in line with previous findings in non-osteoarthritic ligaments [36].

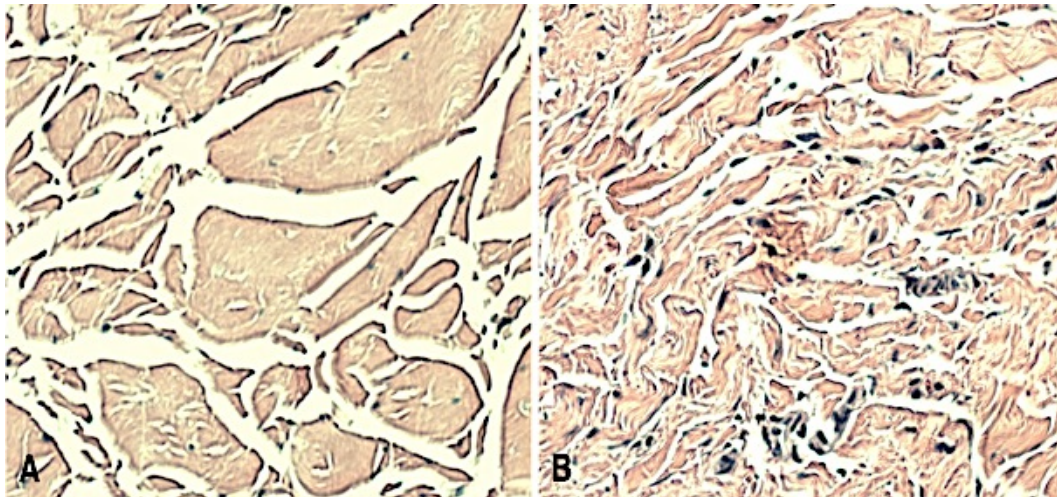


Figure 15. Hematoxylin and eosin stain of (A) DRL and (B) AOL. The DRL is characterized by organized collagen bundle whereas the AOL contains disorganized connective tissue. (Reprinted with permission from Mobargha N et al. Ultrastructure and innervation of thumb carpometacarpal ligaments in surgical patients with osteoarthritis. Clinical orthopaedics and related research. 2014, 472: 1146-54).

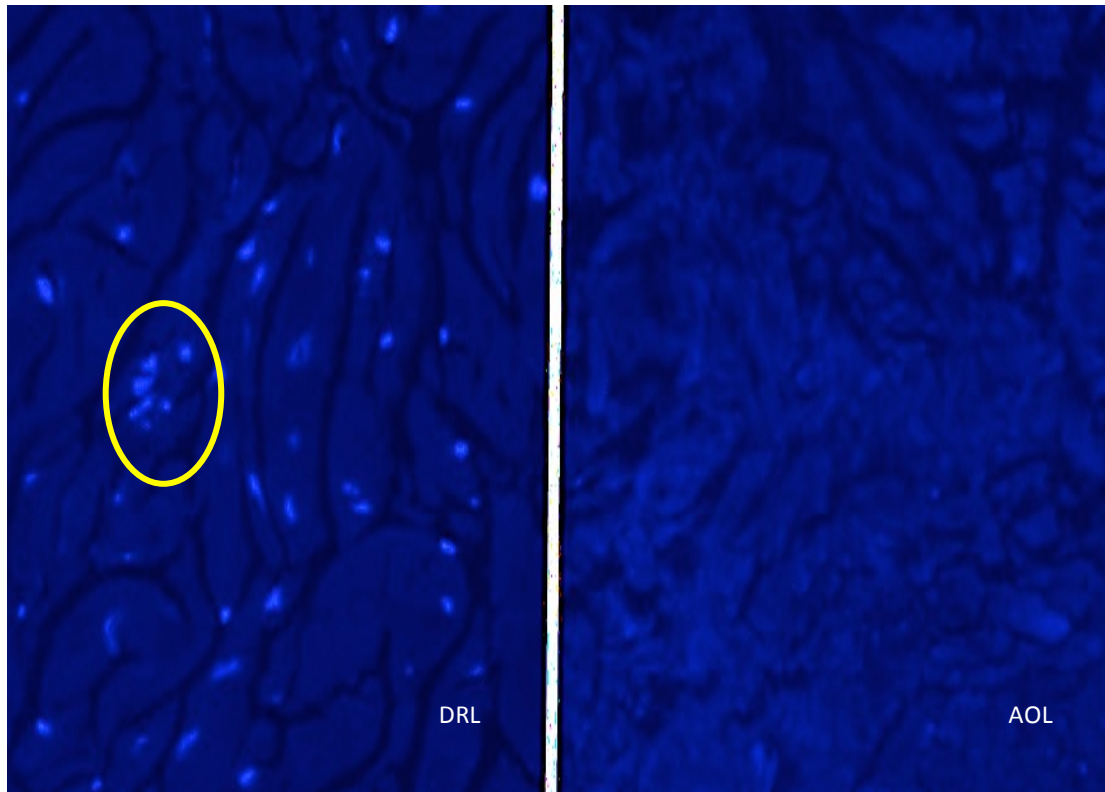


Figure 16. Structural differences between DRL and AOL observed following DAPI labeling. DRL contains a large number of nuclei while AOL is disorganized and distinctively free from nuclei.

5.1.2 Innervation of ligaments

Study I

All of the 22 osteoarthritic CMC1 ligaments examined contained mechanoreceptors. Statistical analysis ($p < 0.001$) demonstrates that the innervation differs between DRL and AOL. There were significantly more mechanoreceptors and free nerve endings found in the DRL. No significant relationship between the innervation and age of patients could be established.

Mechanoreceptors

Immunofluorescence imaging revealed that nerve fibers and mechanoreceptors were most easily detected close to vascular structures, embedded near the ligament's epifascicular regions. Mechanoreceptors were identified in all ligaments. Pacini corpuscles, Ruffini endings and unclassifiable corpuscles were recognized and distinguished from arterioles by triple staining techniques.

5.1.3 Distribution of mechanoreceptors

Study II

In this study, the ligaments were investigated with regard to their proximal and distal portions respectively. This analysis did not provide with any statistically significant mean difference of the innervation of proximal and distal endings of DRL or AOL.

General distribution

A striking observation was the many type V unclassifiable corpuscles (fig. 2) observed in both AOL's and DRL's proximal and distal regions respectively. No significant difference was found when the distal end of DRL was compared to the distal end of AOL. Likewise, no significant difference between the examined ligaments' proximal regions could be detected.

Free nerve endings (fig. 17) were also frequently found in the ligaments, mostly near vascular structures and connective tissue. They were observed in both DRL and AOL. No significance was found when the distal segment of DRL was compared to the distal end of AOL. This was also true for the ligaments' proximal segments.

Only one Pacinian corpuscle was observed in the samples, and was found in the distal DRL. This corpuscle was characterized by its clear p75 immunofluorescence and lamellar corpuscle.

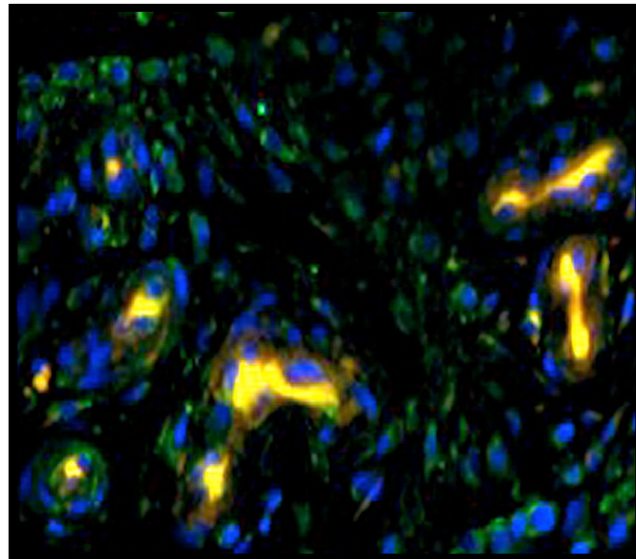


Figure 17. Free nerve endings were frequently identified in both AOL and DRL.

5.2 BIOMECHANICAL STUDY

Study III

The results obtained from isometric loading of individual muscles indicate that certain muscles tested may have an advantageous and stabilizing impact on the joint. Contrarily, other muscles may act as destabilizers by increasing the tendency of dorsoradial subluxation.

Distance between sensors S1 and S2

When DI was loaded, a decrease in the distance between sensors S1 and S2 was observed (fig. 18). This is indicative of a reduction of the radioulnar or horizontal translation of CMC1 and suggests a stabilizing mechanism. Contrarily, an increase in S1-S2 was seen upon loading of OPP, suggesting a radial subluxation of the CMC1.

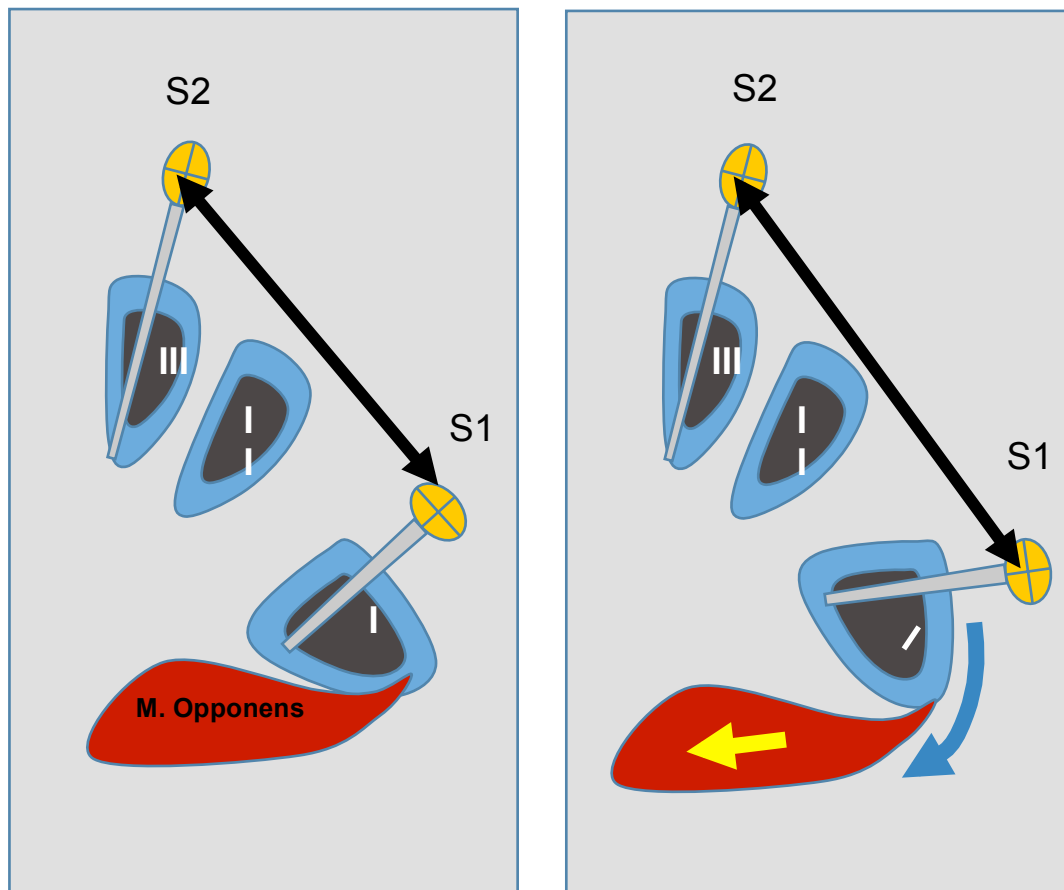


Figure 18. Increase of the distance between sensors S1 and S2. The distance between sensors S1 and S1 is representative of a radial subluxation of the base of the first metacarpal or a pronation, but most likely a combination of both these movements. Courtesy of M Garcia-Elias, MD.

Distance between sensors S1 and S3

When loading the APL, a reduction in the distance between sensors S1 and S3 was observed (fig.19). This distance represents a vertical translation of the sensor S1, and thus a proximal migration of the base of the MC1.

Contrarily, a positive value suggests a combination of MC1 flexion and distal migration. The most clinically relevant finding was that DI was the only muscle that caused an increase in the distance between sensors S1 and S3, thus indicating a stabilizing role to the DI in avoiding dorsoradial translation.

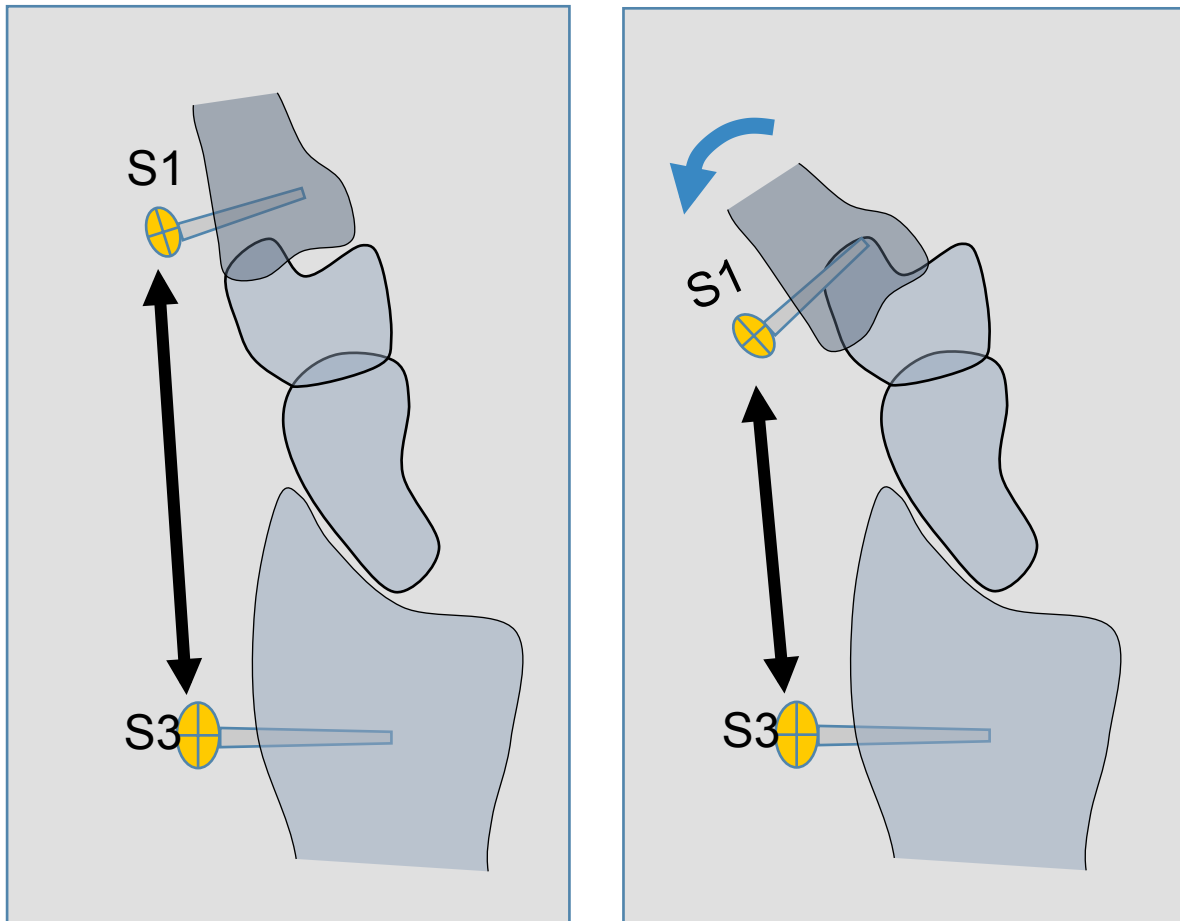


Fig 19. Distance between sensors S1 and S3. Reduction of the distance between sensors S1 and S3, is an indication of proximal migration of MC1, possibly combined with an extension of MC1. Isometric loading of APL reduced this distance most, suggesting possible harmful impact on CMCI. On the contrary, the only muscle that increased this distance was DI. Courtesy of M Garcia-Elias, MD.

5.3 NEUROPHYSIOLOGICAL STUDY

Study IV

The results obtained from EMG analysis of CMC1 muscles following stimulation of DRL revealed significant ($p < 0.05$) changes in the post-stimulus (t_2) activity of DI, APB, APL and EPL during functional CMC1 tasks.

Positive amplitudes represent excitatory muscle activity whereas inhibitory responses are negative in amplitude, as compared to pre-stimulus baseline (t_1).

5.3.1 General observations

Intrinsic muscles

DI demonstrated an early excitatory response during both palmar and key pinch within 40-60 ms following stimulation. During key pinch, DI only presented with excitatory action whereas it was inhibited during the other positions test.

APB reports with only late (> 100 ms) excitatory reactions during palmar pinch whilst this muscle demonstrated an early excitatory response within 40 ms during key pinch followed by mixed activity. APB was solely inhibited during tip pinch.

Extrinsic muscles

Both APL and EPL are rapidly inhibited during palmar pinch. This reaction occurs within 20-40 ms followed by late phase excitations, which suggests co-contraction reactions.

A rapid monophasic reaction was observed in APL during key pinch in form of an excitation, whereas this rapid response was an inhibitory reaction in palmar pinch. Overall, APL was the only muscle to react within 20 ms following stimulation. APL and EPL generally revealed reduction of their amplitudes after stimulation of DRL.

5.3.2 Specific observations

Post-stimulus histograms represent the time after stimulation of DRL. The data is divided into 20 ms time intervals until 500 ms after stimulus (t_2) and is compared to baseline (t_1), 100 ms before the stimulus.

Tip pinch

Interestingly, the post-stimulus reactions during tip pinch reveal a mass inhibition of all muscles investigated (figs. 3 and 20). This response is found around 60ms after stimulus and is the most consistent reaction found in any of the positions studied. Tip pinch presented with most cases of significant amplitudes ($p < 0.05$) throughout the 500 ms run compared to both key pinch and palmar pinch.

Key pinch

An immediate excitatory response is noted in DI, APB and APL during key pinch and implies fast co-contractions, most distinctly found in DI. However, DI became completely inactive after this reaction. The primary excitatory response was followed by inhibitory activity and was first observed in EPL around 200 ms. EPL was almost entirely inhibited during key pinch from 200 ms onwards. APB and APL fluctuated between inhibition and excitation for the entire post-stimulus time interval.

Palmar pinch

Instant inhibition was observed in both APL and EPL from 20-60ms and was followed by excitation of DI at 60-80 ms. After this, DI mainly demonstrated inhibitory activity for the remainder of the time. Co-contractions were lastly observed in APB, APL and EPL from 280-480 ms.

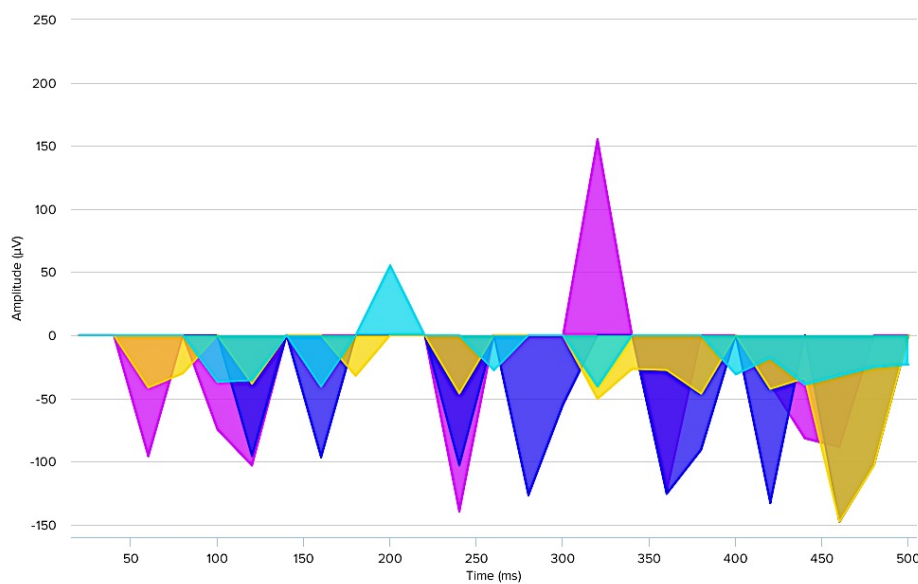


Figure 20. Post-stimulus time histograms for tip pinch. Each column represents statistically significant ($p < 0.05$) median values of amplitudes (Y-axis) for each 20 ms time interval from 0-500 ms (X-axis), following stimulation of the dorsoradial ligament. Inhibitory activity is present and predominant for all muscles throughout tip pinch following stimulation of the dorsoradial ligament.

6 SUMMARY OF RESULTS

The results from study I-IV can be summarized as follows:

- Mechanoreceptors have been identified in the CMC1 ligaments of osteoarthritic patients by immunohistochemical methods.
- The DRL was the ligament in which most mechanoreceptors were identified.
- The innervation of ligaments in non-osteoarthritic specimens differs from patients with CMC1 osteoarthritis, where the latter have a reduction of Ruffini-Pacini innervation and a high frequency of unclassifiable receptors.
- Biomechanical evaluation of the CMC1 joint in isometric conditions suggests that DI has a stabilizing impact on the joint as it decreases dorsoradial translation, whereas APL acts destabilizing.
- Ligamento-muscular reactions were found following stimulation of DRL. Post-stimulus reactions, either inhibitory or excitatory, were observed in DI, APB, APL and EPL after stimulation of DRL.
- Mass inhibitory reactions were observed during tip pinch, whereas other functional CMC1 tasks displayed co-contractions.
- The only muscle to react within 20 ms was the APL, suggesting a primary DRL-APL ligamento-muscular reflex for joint control.

7 DISCUSSION

The findings of this thesis collectively suggest that the CMC1 joint is a joint with proprioceptive and neuromuscular functions. Therefore, this thesis proposes the idea of the CMC1 joint to be viewed as a synovial organ, to which all aspects of neurosensory, biomechanical, neuromuscular functions are crucial for proper joint control and stability.

Consequently, any alteration of the integral parts of this complex organ may result in disrupted ligamentous, muscular or reflex characteristics, potentially rendering the joint susceptible to osteoarthritis [54,67].

7.1 PROPRIOCEPTIVE FEATURES

The median, ulnar and radial nerve all innervate the CMC1 joint. This contributes to the complexity of this joint as all three nerves to the hand contribute to thumb function.

As declared in previous sections, the DRL of osteoarthritic patients presented with significantly more mechanoreceptors than the AOL. This observation is consistent with previous research in the field, in which immunohistochemical studies of wrist ligaments revealed that their dorsal regions were more innervated than the volar structures [82,84].

Additional investigations have been made on CMC1 ligaments. These studies are based on non-osteoarthritic CMC1 joints, in which the dorsal ligaments were also found with rich innervation compared to the volar ligaments [35-37]. However, the findings from those studies differ significantly compared to this investigation on the matter of actual mechanoreceptor populations.

In the prior studies made on non-osteoarthritic CMC1 ligaments, the Ruffini ending was the most frequent type of mechanoreceptor identified, whereas the unclassifiable corpuscles and free nerve endings were dominant in this investigation. Other mechanoreceptors, such as Ruffini endings and Pacinian corpuscles were not as frequently found in the osteoarthritic ligaments, as compared to non-osteoarthritic specimens. These two types of mechanoreceptors adapt rapidly to joint position, rotation and joint acceleration/deceleration respectively. This reduction of Ruffini endings and Pacinian corpuscles in osteoarthritic ligaments, suggest that osteoarthritic ligaments may have inferior proprioceptive and neuromuscular abilities. This since afference from mechanoreceptors is a prerequisite for proper joint control and stability and conduce continuous adaptation to movement [54,85]. Similar results have been observed in studies on the mechanoreceptor populations of patients with hip osteoarthritis. The hip ligaments of these patients revealed that the general ligamentous innervation was significantly altered as compared to healthy hips and exhibited a predominance of free nerve endings [60].

A plausible explanation for the divergent report between non-osteoarthritic ligaments compared to osteoarthritic specimens may lie in the osteoarthritic process itself. The abundance of unclassifiable mechanoreceptors identified, may be the result of continuous traumatic loads, trauma or modification of the receptors due to complex inflammatory processes, which occur as osteoarthritis evolves. A similar course of development is noted in the hand where hyperplastic changes of Pacinian corpuscles have been observed, a condition often associated with local pain and history of trauma [86,87].

Another group of mechanoreceptors frequently identified in our material, were the free nerve endings. The free endings are likely involved in inflammatory activity, as they are carriers of vesicles containing inflammatory elements, such as substance P [88,89]. With this in mind, it is clear that the role of free nerve endings and their role in the course of osteoarthritis progression needs further investigation, since noxious input, inflammation and pain are common symptoms amongst osteoarthritic patients.

7.2 NEUROMUSCULAR MECHANISMS

Neuromuscular deficiency and weakness often precede the onset of osteoarthritis [21,67]. Hence, patients with CMC1 osteoarthritis often present with poor neuromuscular capacity and instability of the joint [10]. With this in mind, we investigated the neuromuscular mechanisms of the CMC1 joint in normal conditions, which provides for the foundation of future investigations of osteoarthritic CMC1 joints.

Previous investigations of CMC1 musculature have reported the beneficial effects of DI as a stabilizer of the thumb, which is in agreement with our findings [16,90,91]. However, because DI does not actually span across the CMC1 joint, it has not typically been valued as a key component of the CMC1 joint [16]. Yet, this muscle possesses the capacity to stabilize the CMC1 joint by reducing the tendency of dorsoradial subluxation, a condition noted in patients with severe CMC1 osteoarthritis. In concordance with this, the favorable properties of DI have been noted in patients with CMC1 osteoarthritis; patients who were treated specifically with DI targeted exercises demonstrated less pain and disability, compared to patients with conventional CMC1 osteoarthritis exercise [92].

On the contrary, we identified APL as the muscle with more disadvantageous effects on CMC1. When isometrically loading the APL, we noted that the CMC1 was compressed and dorsoradially translated. This type of load is not beneficial for joint stability and joint alignment as it may contribute to the onset of osteoarthritis. In fact, APL has previously been acknowledged for its detrimental role and it has been recommended to perform tenotomy of auxiliary tendons in early stages of CMC1 osteoarthritis [93].

Interestingly, the APL was the only muscle to react immediately (within 20 ms) following stimulation of the DRL in healthy subjects. These reactions indicate a particular importance in ligamento-muscular control of the joint. Since the APL is disadvantageous in established CMC1 osteoarthritis, a disturbance of this ligamento-muscular reflex may be hypothesized. Future EMG investigations should thus include subjects with known CMC1 osteoarthritis.

Besides proper muscular equilibrium, joint innervation and the integration of joint afference, e.g., ligamento-muscular pathways, are essential for neuromuscular control and joint stability [53,54]. It has been established that during the process of aging, a natural denervation process occurs [69, 70]. This may in part play a role in the complex etiology of osteoarthritis and its increasing prevalence with age, as denervation may result in ineffectual neuromuscular mechanisms and proprioceptive functions [94-96].

Previous research has proposed that joint denervation may in fact intensify cartilage degeneration, alter joint reflexes and thus result in osteoarthritis [71, 72]. Joint innervation is also central as denervated joints lose their free nerve endings and vessels and consequently obtain disadvantageous healing abilities [94]. Consequently, this also increases the risk for osteoarthritis to develop [96,97].

Yet, the dynamics and interplay between CMC1 muscles and innervated CMC1 ligaments have not been previously explored. Our investigation has for the first time established a ligamento-muscular pathway between CMC1 muscles and a CMC1 ligament, namely the richly innervated DRL. Our data adds to present knowledge about possible protective ligamento-muscular functions [85,98-100].

One interesting finding of our study, was the identification of a mass inhibition of muscular activity during tip pinch. This position is especially straining for the CMC1 joint as large force amplification, equivalent of 1200%, occurs at the CMC1 joint during tip pinch [47]. Moreover, tip pinch is a particularly biomechanically challenging position as the CMC1 is positioned at mid-axis [47] and engages all four muscles investigated in our material, in order to maintain joint equilibrium. Therefore, the major inhibition of muscle activity noted during tip pinch is indicative of a protective ligamento-muscular response by self-limitation of excessive joint load.

Another important finding that supports the idea of joint protective ligamento-muscular reactions was observed during key pinch. After stimulation of DRL, a rapid co-contraction was identified just after 20-40 ms. This type of ligamento-muscular reaction is similar to a monophasic reaction, and suggests the presence of a rapid monosynaptic spinal reflex [85,101].

We were also able to identify co-contractions during all three functional CMC1 tasks investigated, as have been described in other joints [102-104]. The co-contractions facilitate joint stability by concurrent activation of agonist and antagonistic muscles and generate joint stiffness, which further promotes joint stability.

Overall data obtained from our investigations demonstrate and confirm that proprioceptive and neuromuscular functions are present in CMC1 and that these functions are in all likelihood of importance for proper joint functions. Further work is required to fully understand these mechanisms and their probable course of transformation during the onset and progression of CMC1 osteoarthritis.

7.3 LIMITATIONS

The studies in this thesis have certain limitations, which will be considered and discussed in the following section.

7.3.1 Immunohistochemical methods

There are several known limitations to immunohistochemical methods and these can be divided into pre-analytic, analytic and post-analytic limitations (fig. 21).

Pre-analytical limitations

Once removed, it is important to fixate the tissue e.g. in formalin and paraffin promptly to avoid autolysis [105,106]. The paraffinization and deparaffinization procedures of the samples also contribute to unwanted exposure of the tissue. On the other hand, our samples were fresh paraffin-embedded, rather than fresh-frozen tissue as used in previous studies [35-37]. This reduces the exposure of the tissues and reduces the risk for artifacts to form.

Another pre-analytic phase of importance is the sectioning of the ligaments. This step was performed by a microtome that segmented the samples into five μm sections. This may result in cases where important structures are lost if divided as they may be localized in the transected area. However, this matter is still existent even if larger sections would have been used.

Analytical limitations

The analytical aspects of immunohistochemistry are much dependent on the protocol, antigen and antibodies used, as well as proper use of controls. The antibodies used in our studies have been used extensively before [35-37].

To avoid possible sources of error a standardized protocol is necessary. This is also true for the staining procedures themselves, as they are shown to be more reproducible when using immunohistochemistry through automated systems [107]. This type of automated systems requires large number of samples to be feasible. Therefore, this was not in the scope of our studies, as we used smaller sample sizes. Moreover, the use of optimal antibodies and the characteristics of antigens, are key factors in the reliability and accuracy of immunohistochemical methods.

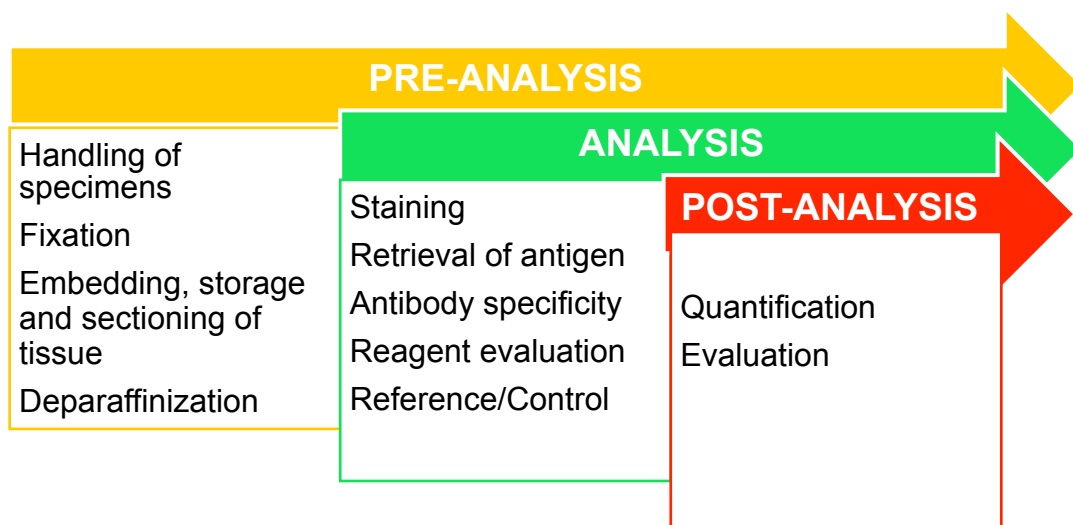


Figure 21. Limitations to immunohistochemical methodologies. Adapted from US Biologic Stain Commission 1991 [108].

Post-analytical limitations

Post-analytical limitations of immunohistochemical studies are often regarding the interpretation of the results. In our investigations, we used a semi-quantitative scoring system, based on previous published studies on microscopic analysis of human ligaments [36,58,84,109]. Manual interpretation of immunohistochemical stains, are probably not as reliable as automated systems [110-112]. The semi-quantitative analysis used is often labeled as a blunt method of data evaluation, but remains the preferred method of choice until completely standardized methods of immunohistochemistry are introduced [106]. Lastly, another post-analytic challenge associated with immunohistochemistry and immunofluorescent labeling, is the photo bleaching that occurs due to cumulative light exposure of the prepared tissue.

7.3.2 Biomechanical assessment

The biomechanical model used in study III is a simplified representation of the complex and multifaceted CMC1 joint. Naturally, a complete reconstruction and deconstruction of the anatomical and biomechanical conditions of CMC1 is not feasible *ex vivo*. The model used has kept the joint intact, but it is still difficult to assess all aspects of muscular control of this joint, which in real life encompasses both dynamic, isotonic and isometric muscle activity. Furthermore, our study set-up was unable to include aspects of thumb supination and pronation, which also play a role in the dynamics of CMC1 function. Nevertheless, the FASTRAK® motion tracking device is reported as a reliable method to evaluate a three-dimensional analysis of joint motion [113-118], as opposed to e.g. the use of manual goniometer which is a device known to be disposed to sources of measurement error.

7.3.3 Electromyographic procedures

Factors that commonly may impact EMG signals are often different forms of electrical noise interference. Noise interference stems from either electromagnetic devices, also known as ambient noise interference, or it can also be due to noise generated from the electrical device used [119]. Other sources of electrical noise that can disturb EMG signaling include motion artifacts due to interference of the electrode or the cable itself. There are also extrinsic causes of disturbance of EMG signals, which can vary due to the type of electrode used and the positioning of the electrode. Intrinsic causes, which may disturb EMG signaling, are primarily physiological issues such as blood flow or fiber type and localization [119,120].

A weakness of our electromyographic study was that we did not include all muscles, which control CMC1. However, the muscles included were selected for representation of both intrinsic and extrinsic muscle activity in addition to their varying innervation by the median, radial and ulnar nerves. In addition, the muscles were selected to reduce the possibility of crosstalk, which is known as a frequent source of error in electromyographic procedures [121]. Additional steps taken to reduce the risk of crosstalk were use of a reference electrode as well as meticulous skin preparation and visual inspection of signals to ensure that signals from the different muscles were not symmetrical.

Another imaginable source of uncertainty was the possible interference of pain afferents in the examined area. To minimize the risk of such events, the stimulus applied to DRL was consistently below the pain threshold. Consequently, the risk of involvement of nociceptive A δ - or C-fibers was eliminated, and paved the way for detection of A β -afferents of mechanoreceptors present in the DRL [1,57,122]. In addition, none of the participants experienced pain during the stimulus.

7.4 CLINICAL RELEVANCE

The clinical implications based on this thesis, may serve to aid in the prevention and treatment of CMC1 instability and osteoarthritis. The results indicate an association between impaired proprioceptive and neuromuscular functions, abnormal joint load and osteoarthritis, in agreement with previous research [56,123,124]. Traditional rehabilitation often aims at improving muscle strength and when this is combined with neuromuscular exercise, positive outcomes such as pain reduction and improved functions have been observed [125].

Previous researchers have demonstrated that if effective proprioceptive exercises are used to target ligament injuries, in e.g. the ankle and the knee, the need for surgery will decrease [126-128]. A specific type of neuromuscular exercise, named perturbation exercise, has been employed for the rehabilitation of the anterior cruciate ligament injuries in the knee, with positive outcomes as patients improved their gait patterns and knee function [125,129,130]. Also in the shoulder, another joint highly subjected to instability with dependency on proper proprioceptive and neuromuscular functions, neuromuscular exercises are also recognized as key components in the restoration of joint control and joint function [131].

Both surgical and non-surgical treatments of CMC1 osteoarthritis aim at improving muscle strength and range of movement whilst reducing pain, stiffness, instability and joint incongruency. This is especially important, as the biconcavo-convex shape of the CMC1 joint does not provide for optimal intra-articular stability and support.

Conventional non-surgical treatments of the thumb include pain medication, orthotic treatments, strengthening exercises and joint protection education [132,133]. The purpose of dynamic, neuromuscular exercise of CMC1 is to ensure stability and precision throughout the large range of motion, which this joint is capable of.

Central to this discipline is the importance of DI strengthening exercises, combined with restoration of the muscular equilibrium of CMC1's intrinsic and extrinsic musculature [92]. This resonates with the findings observed in our biomechanical study [3], in which the DI counteracts dorsoradial shift. However, much remains to be investigated on the topic of proprioceptive re-education of the hand and the CMC1 joint, as this is still a subject without real consensus [134].

As we have confirmed the presence of a ligamento-muscular reflex following stimulation of the DRL, we advocate that this should also be taken into consideration during surgical treatments of CMC1 by employing nerve-sparing techniques. In cases with carpal instability, ligament insufficiency has reportedly been treated with electro thermal shrinkage through arthroscopic approach [135,136]. Although these reports present with positive outcomes, this type of treatment should be used with caution, whether it is for the treatment of carpal instabilities or CMC1 instability. This is based on the notion that this treatment may cause irreversible damage of mechanoreceptors and free nerve endings that, according to the findings of this thesis, are central for optimal joint stability and joint functions.

In conclusion, neuromuscular and proprioceptive features of CMC1 should be taken into consideration during treatment of CMC1, whether surgically or conservatively. As such, we propose early post-operative rehabilitation and perhaps even pre-surgical proprioceptive strategies to optimize surgical outcomes.

7.5 FUTURE PERSPECTIVES

In order to understand the pathophysiology of this intricate joint, one needs to outline normal physiological conditions, which partially have been described in this thesis. The conclusions regarding the proprioception and neuromuscular stability of CMC1 as presented herein, are steps towards deciphering the pathophysiology of this complex thumb joint. Still, there are still many questions that remain unanswered but these are outside the scope of this thesis.

Research questions that could be asked include the function and exact role of unclassifiable receptors. Are these receptors degenerated or injured mechanoreceptors with new functions? Or do they represent non-functional neural remnants? Have these receptors been present in osteoarthritic patients all along, making them susceptible to osteoarthritis, or are they a result of the osteoarthritic process?

To characterize these receptors further, one could design a set-up in which different stages of osteoarthritis are compared and assessed. In a best-case scenario, one could examine whether a linear relationship is present between the various stages of osteoarthritis and the prevalence of unclassifiable receptors, as opposed to the present comparison of end stage osteoarthritis to normal specimens.

Further work is required to evaluate the beneficial effects of neuromuscular exercise of DI as a treatment option in early CMC1 osteoarthritis. A possible way to do so would be to compare patients with neuromuscular exercise of DI to those with traditional strengthening exercises and patients who receive no treatment. This can also be evaluated with regard to surgical intervention; do patients who undergo dynamic exercise of DI prior to surgery have better surgical outcomes, as noted in patients who undergo reconstruction of the anterior cruciate ligament of the knee [137-139]?

To develop a full picture of the neurophysiological conditions in CMC1, additional studies will be needed to characterize the ligamento-muscular patterns in patients with CMC1 osteoarthritis. In addition further research should be undertaken to investigate the role of maintained innervation of CMC1, as some surgeons advocate joint denervation as treatment in osteoarthritic patients. But how does this affect the proprioceptive function of the joint?

If one were to widen the perspectives further, the methods used in this thesis can also be applied to investigate other joints or neuromuscular pathologies, such as affected joint and/or muscle functions following nerve palsy or injury.

8 CONCLUSIONS

This thesis began by describing the characteristics and innervation of CMC1 ligaments in osteoarthritic patients, followed by a biomechanical assessment of CMC1 muscles. Finally, electromyographic observations of CMC1 and its ligamento-muscular relations revealed the presence of protective ligamento-muscular pathways. Some of the principal issues and conclusions are provided below:

- CMC1 ligaments are essential for proper joint stability and control, not simply due to their mechanical support, but also because they embody sensorimotor and neuromuscular qualities.
- The DRL is a robust ligament with ample innervation, whereas AOL is thin and hypocellular. The most common type of mechanoreceptor found in DRL, was the unclassifiable receptor. This finding differs from specimens investigated without CMC1 osteoarthritis, and may be an implication of altered sensorimotor features as a result of osteoarthritis, natural senescence, or due to post-traumatic changes.
- Biomechanical assessment of CMC1 in isometric conditions revealed that certain CMC1 muscles may act as stabilizers of the joint by preventing dorsoradial translation, whereas other muscles actually advance joint malalignment.
- Functional evidence for the proprioceptive and neuromuscular functions of the CMC1 joint was established by the electromyographic observations of CMC1 muscles after stimulation of DRL. The ligamento-muscular reflex responses identified likely serve to promote joint stability.
- Overall, these findings support the view that the CMC1 joint is not merely a biomechanical unit, but a joint with complex proprioceptive and neuromuscular functions that are needed to maintain the stability of this inherently incongruent and multifaceted joint.
- Clinically, these results advocate nerve-sparing surgical techniques and highlight the need to further explore the effects of early rehabilitation following trauma or surgery. By doing so, neuromuscular and proprioceptive functions are preserved and the onset of osteoarthritis may be delayed or at its best, prevented.

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